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RESEARCH ARTICLE

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Platelet aggregometry cannot identify uremic platelet dysfunction in heart failure patients prior to cardiac surgery

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Background: Patients with heart failure often have concomitant renal disease which can result in uremic platelet dysfunction. Determining whether uremia has affected platelets by platelet aggregometry can be challenging in these patients since they are often on antiplatelet medications. This study was undertaken to determine if platelet aggregation studies could identify heart failure patients at risk for uremic bleeding prior to cardiac surgery.

Methods: Platelet aggregation studies from three groups were studied and compared: 17 heart failure patients with mild to moderate renal impairment, 17 heart failure patients without renal abnormalities and 17 healthy volunteers.

Results: Platelet aggregation was severely impaired in both heart failure groups with and without renal abnormalities compared to healthy controls, and there were no significant differences in platelet aggregation in response to any of the agonists. There was a pan-decrease in platelet aggregation to all agonists in all heart failure patients.

Conclusion: Platelet aggregometry does not appear to be useful in measuring platelet dysfunction in heart failure patients with mild to moderate renal impairment.

KEYWORDS

aggregation, cardiac, dysfunction, platelet, surgery, uremia

1 | INTRODUCTION

Platelet dysfunction due to uremia is a well-known complication of renal failure patients. The pathophysiology of uremic platelet dysfunction is multifactorial with defects in adhesion, secretion, and aggregation. Some biochemical factors implicated include uremic retention solutes such as guanidinosuccinic acid,[1-3] phenols, urea, and creatinine.[4-7] There is still no unifying theory that accounts for all the platelet defects. Accumulation of uremic toxins may inhibit platelet aggregation and result in bleeding. The threshold for azotemia for which bleeding occurs, however, has yet to be determined.

At our institution, we routinely perform a pre-operative hemostatic risk assessment for most pre-op cardiac surgery patients as part of our Hemotherapy clinical service.[8] Although not all inclusive, the majority of surgeries we perform bleeding risk assessments for include orthotopic heart transplants (OHTs), left ventricular assist devices (LVADs) and aortocoronary bypass (ACB). Quite often these patients have concomitant renal dysfunction with underlying heart disease. It is therefore desirable to identify those renal failure patients at risk for uremic bleeding during and after surgery by testing platelet function since this will allow adequate time to prepare appropriate therapies (cryoprecipitate, desmospressin, prothrombin complex concentrates) targeted at controlling uremic bleeding.

Testing platelet function is a complicated task given that the majority of heart failure patients are routinely on antiplatelet therapy. Drugs such as aspirin strongly inhibit arachidonic acid and other platelet

agonists to some degree, thus confounding the results of platelet aggregation studies used to evaluate the degree of uremic-induced platelet dysfunction. Despite its limitations, platelet aggregometry may still have some usefulness to detect platelet abnormalities caused by uremia even when a patient is taking aspirin.

This study was conducted to investigate the utility of platelet aggregation studies in a cohort composed of heart failure patients prior to cardiac surgery and to determine what degree of renal impairment would result in abnormal platelet aggregometry.

2 | METHODS

2.1 | Study design

A retrospective chart review was performed between January 2013 and December 2015 to identify cardiac surgery patients with and without renal impairment who had platelet aggregation studies performed. Mild to moderate renal impairment was defined as a serum creatinine >1.5 mg/dL. Subjects were excluded if they were taking antiplatelet drugs (besides aspirin), had thrombocytopenia or a thrombotic condition, or were actively bleeding. Demographic data and clinical data were collected along with laboratory values such as CBC, creatinine, BUN, and eGfr. This study was approved by the Committee for the Protection of Human Subjects, the Institutional Review Board for the University of Texas Medical School at Houston (HSC-MS-13-0440).

2.2 | Platelet aggregation assay

Light transmission aggregometry (LTA) was performed using whole blood on the Bio/Data PAP-4 aggregometer (Bio/Data Corporation, Horsham, PA, USA). Blood was collected into tubes with 3.2 g of sodium citrate (sodium citrate, whole blood ratio 1:10). Platelet rich plasma (PRP) was prepared by centrifugation of this anticoagulated blood at 180 g for 10 minutes. Collection/transport of specimen and centrifugation were all prepared at room temperature. Platelet aggregation was determined by measuring the change in the optical density (light transmittance) of stirred PRP after addition of the aggregating agent to the aggregometer cuvette. Aggregating agonists included adenosine diphosphate (ADP) at final concentrations of 2.5 and 50 µmol/mL, ristocetin 0.75 and 1.5 mg/mL, epinephrine 5.5 and 11 µmol/mL, arachidonic acid 2.5 and 5 mg/cL, and collagen 5 µg/mL. Results are expressed as the percent change in light transmittance after agonist addition. A normal platelet control was performed with each aggregation study. Abnormal aggregation in response to an agonist was considered present if there was <60% aggregation.

2.3 | Statistical analysis

Light transmission aggregometry and laboratory parameters are expressed as mean values. The primary objective was to compare LTA and laboratory parameters between heart failure patients with and without renal abnormalities, and hence our sample size calculation is based on two-sample *t* tests. With 17 patients in each group, we have 80% power to detect a 1.4*standard deviation (SD) difference between groups, with Bonferroni correction to adjustment for multiple comparisons and a 5% type-I error rate. For example, assuming that SD of BUN is 13, we have more than 80% power with 17 patients in each group to detect a mean difference larger than 18.2 between two groups.

Also, the sample size of our retrospective chart review has been chosen based on the feasibility and exploratory nature of the study.

One-way analysis of variance (ANOVA) was conducted to compare the means of three groups. For pairwise comparison, that is, heart failure patients without abnormalities vs control and patients with renal impairment vs control, two-sample *t* tests (one-sided), which test whether the former has lower mean than the latter, were conducted. To assess whether there exists significant mean difference between heart failure patients with renal impairment and without abnormalities, two-sample *t* tests (two-sided) were conducted. Statistical analyses were performed using R software (version 3.2.3, https://www.r-project.org/about.html).

3 | RESULTS

Fifty-one subjects (17 healthy controls, 17 heart failure patients with abnormal renal function, and 17 heart failure patients with normal renal function) during the study period were evaluated. Demographic and clinical characteristics are presented in Table 1. Both heart failure patient groups were comprised of approximately equal numbers of males and females. The majority of patients (90%) were taking aspirin at a dosage of 325 mg daily. The average BUN and creatinine were significantly elevated and eGFR was significantly decreased in the renal dysfunction group when compared to the normal renal function group, however, there were no significant differences in the hematologic parameters (Table 2).

Both patient groups had significantly decreased aggregation with all agonists when compared to normal controls. Although the mean ristocetin (high dose) induced aggregation was lower in the renal impairment group, there were no significant differences in platelet aggregation between the normal renal function group and renal dysfunction group in response to any of the agonists including ristocetin.

To explore the effect of severe renal dysfunction beyond the range considered in this study, we examined the platelet function in a heart failure patient with severe uremia. Figure 1 documents four consecutive platelet aggregation studies in a 48-year-old patient on aspirin therapy to demonstrate the extent of azotemia required to see significant changes. For this patient, the eGFR, creatinine and BUN levels were 9 mL/min, 9 mg/dL, and 32 mg/dL, despite being on hemodialysis. All agonists except high dose ADP showed severely depressed aggregation with transmission <10%.

4 | DISCUSSION

This study was performed to determine the utility of platelet aggregation studies as a pre-operative screening tool for uremic platelet

TABLE 1 Subject demographics and clinical characteristics of all heart failure patients

Parameters	Normal renal function (N=17)	Renal dysfunction (N=17)
Age (y)	53±14	55±15
Female gender (%)	12%	0%
Underlying heart disease		
Ischemic cardiomyopathy (%)	59%	39%
Non-ischemic cardiomyopathy (%)	35%	50%
Familial carciomyopathy (%)	0%	11%
CAD Stable angina (%)	6%	0%
NYHA class		
Class I	6%	0%
Class II	6%	0%
Class III	35%	22%
Class IV	53%	78%
Surgery to be performed		
LVAD	70%	89%
LVAD and RVAD	6%	11%
LVAD exchange	12%	0%
ACB	6%	0%
Mitral valve	6%	0%
Overt liver disease or cirrhosis	0	0
Renal disease		
Acute	—	47%
Chronic	—	16%
Acute and chronic	—	37%
Antiplatelet medications		
ASA and heparin	30%	35%
ASA only	65%	65%
Heparin only	0%	0%
ASA and nitroglycerin	5%	0%
Patients receiving dialysis	0%	16%

LVAD, Left Ventricular Assist Device; RVAD, Right Ventricular Assist Device; ACB, Aortocoronary bypass; NYHA, New York Heart Association; ASA, Aspirin.
Plus-minus values are means±standard deviations.

dysfunction in patients with heart failure. Since the majority requires antithrombotic therapy to prevent coronary ischemic events, platelet aggregometry results in this patient population are always abnormal and difficult to interpret. However, even in the context of aspirin use, we hypothesized that platelet aggregometry might still be useful in detecting an additional insult to platelets caused by uremia.

As expected in our study, frequent aspirin use in heart failure patients with and without renal impairment resulted in abnormal platelet aggregometry. All agonists with the exception of ristocetin 1.5 mg/mL were significantly affected with <35% transmission. However, the superimposed effects of renal dysfunction did not result in further detectable decreases in platelet aggregation with the agonists studied. It is possible that the degree of kidney damage and uremia was not severe enough to result in declines in platelet function. The average BUN and eGFR in the abnormal kidney function group was 56.5 mg/dL and 43.7 mL/min. This level of eGFR correlates only with moderate kidney damage (stage 3), while values between 15 and 29 mL/min and <15 mL/min are considered to be severe and kidney failure, respectively. Thus, we identified an additional patient with severe kidney damage on aspirin therapy and studied consecutive platelet aggregation studies to test the idea that uremic platelet dysfunction could be detected in extreme uremic cases. For this patient, the eGFR, creatinine and BUN levels were 9 mL/min, 9 mg/dL, and 32 mg/dL, despite being on hemodialysis. The patient was on no other medications besides aspirin that could cause decreased platelet aggregation. On the basis of the results from Table 2 of this study, we would have expected a drop in transmission of close to 35%. Instead, the platelet aggregation study was found to have aggregation of <10% with most platelet agonists. Since there were no other factors besides aspirin that could have caused such a drastic decline, the severity of the patient's kidney disease was likely the extra insult to platelet dysfunction. Although this was a sample size of one, it suggests that significant renal impairment must occur before detecting additional abnormalities in platelet aggregation for a patient taking aspirin.

Current literature is varied on BUN or creatinine levels that correspond to platelet dysfunction. Ho and colleagues found poor correlation between calculated GFR and the skin bleeding test (SBT) and no correlation with serum creatinine or urea.[5] Furthermore, there was no correlation between abnormal whole blood platelet aggregometry (WBPA) and the degree of uremia. In their cohort, renal failure was defined as GFR <30 mL/min using the Cockcroft Gault equation.[5] Steiner et al.[7] only found correlation between platelet aggregation by collagen to BUN/creatinine and bleeding time but no correlation with ADP or epinephrine. Patients in Steiner's study were classified either with severe uremia (BUN > 102) or mild uremia (BUN < 102).[8] An additional study by Brophy et al.,[9] found a positive correlation between bleeding time and serum creatinine but no relationship with BUN. Disagreement between studies is likely due to lack of standardization of platelet aggregometry from lab to lab since different concentrations are used for each of the agonists. In addition, the definition of uremia varied from study to study with some using GFR and others using BUN. In the age of hemodialysis, eGFR, BUN and creatinine will all improve somewhat so it is difficult to assess the degree of uremia with these parameters. In our own clinical practice, we have found that hemodialysis has the greatest impact on BUN followed by creatinine and eGFR. Perhaps eGFR, then is a better tool to estimate uremia, but this theory remains to be tested.

An unexpected finding for this study was an across the board decrease in platelet aggregation to all agonists in both groups of heart failure patients. Most patients were on aspirin, which should have resulted in decreased responses only to the secondary wave of ADP and arachidonic acid. The patients were not taking any other medications (beta-blockers, diuretics, nitroprusside, angiotensin-converting enzyme inhibitors, etc.) that could have caused a pan-decrease in

TABLE 2 Laboratory results, mean ± SD

Parameter	Heart failure/normal renal function (N=17)	Heart failure/renal dysfunction (N=17)	*P*-value	Healthy controls (N=17)
BUN (mg/dL)	24.29	50.29	<.001	—
Creatinine (mg/dL)	1.26	2.70	<.001	—
eGfr (mL/min/1.73 m2)	68.29	33.59	<.001	—
Hb (g/dL)	11.19	10.93	NS	—
HCT (%)	34.09	33.26	NS	—
Plt (×103/mL)	225	204	NS	—
Platelet aggregation				
Arachidonic acid 5 mg/cL (%)	9.47	11.76	NS	79.47
Arachidonic acid 2.5 mg/cL (%)	10.35	23.29	NS	81.18
ADP 2.5 µmol/mL (%)	28.59	27.18	NS	77.06
ADP 50 µmol/mL (%)	66.12	68.59	NS	81.41
Collagen 5 µg/mL (%)	34.94	36.88	NS	80.82
Epinephrine 11 µmol/mL (%)	34.94	33.29	NS	80.00
Epinephrine 5.5 µmol/mL (%)	29.82	32.12	NS	76.94
Ristocetin 1.5 mg/mL (%)	77.18	77.76	NS	88.35
Ristocetin 0.75 mg/mL (%)	6.82	7.00	NS	6.47

BUN, Blood Urea Nitrogen; eGfr, Estimated Glomerular Filtration Rate; Hb, Hemoglobin; HCT, Hematocrit; Plt, Platelets; ADP, Adenosine diphosphate. *P*-values compare normal to abnormal renal function.

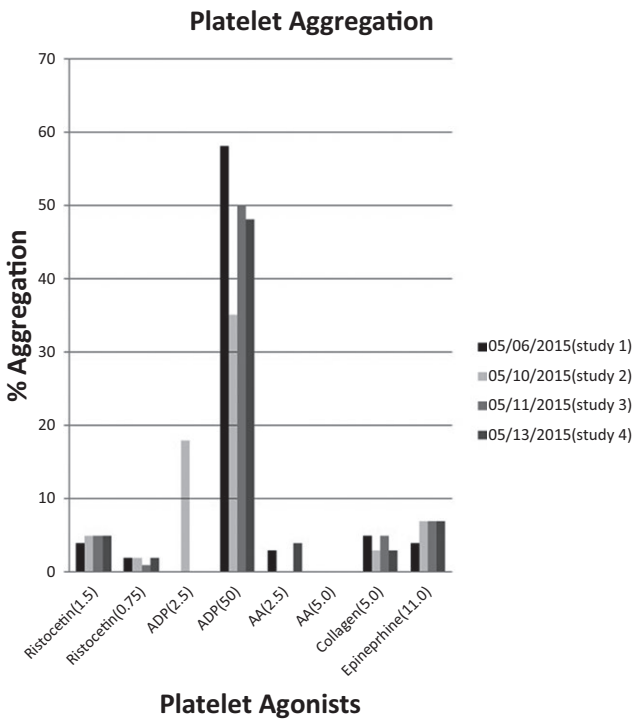

FIGURE 1 Platelet aggregation studies for a patient with severe renal failure

aggregation. It is interesting to speculate that these findings might be related to a neurohumoral adaptation of increasing vasoactive substances. However, these results are in contrast to those found by de Meirelles et al.: in their group of moderate CHF patients, collagen-and ADP-induced platelet aggregation were present, suggesting a role for platelets in the prothrombotic state. In their cohort, patients were not taking aspirin but the majority wasreceiving beta-blockers, diuretics and angiotensin-converting enzyme inhibitors.[10]

The limitations of the current study include its small sample size and retrospective design. Also, platelet aggregation studies were not performed at the time of an acute bleeding episode, but rather during a pre-operative assessment. The ability to test the utility of platelet aggregation studies in predicting peri- and post-operative bleeding was not possible because of the much larger sample size that would have been needed from many other confounding variables. We were interested in measuring uremic platelet dysfunction to determine who would be candidates for specific agents like cryoprecipitate and desmopressin.

In summary, our study highlights the limited utility of platelet aggregometry for detecting platelet dysfunction in heart failure patients with mild to moderate renal impairment. Future studies will need to investigate the effect of severe renal dysfunction and its effect on platelet aggregation studies.

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